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Isolation and Serotyping of Porcine Rotaviruses and Antigenic Comparison with Other Rotaviruses†

EDWARD H. BOHL,* KENNETH W. THEIL, AND LINDA J. SAIF

Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio 44691

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Seven rotavirus strains were isolated in cell cultures from the intestinal contents of piglets with diarrhea. MA104 cells with pancreatin in the cell culture medium was the host system of choice for virus isolation and replication. A cell culture immunofluorescence test in which MA104 cells were used in microtiter plates was very effective for detecting and assaying rotaviruses. A plaque reduction neutralization test, cross-protection studies in gnotobiotic pigs, and electrophoresis of rotaviral double-stranded RNA were used for comparing viruses. Three strains produced plaques on initial isolation attempts, replicated well in cell cultures, and were antigenically very similar. We suggest that these three strains be considered porcine rotavirus serotype 1, with The Ohio State University (OSU) strain serving as the prototype. The OSU strain was distinct from bovine, simian, canine, and human (Wa and M) rotaviruses by plaque reduction neutralization. Four strains did not produce plaques on initial isolation attempts, were difficult to adapt to cell cultures, and were related to each other but were distinct from the serotype 1 strains. We suggest that the Gottfried (G) strain be tentatively considered as a prototype for porcine rotavirus serotype 2. The G strain was antigenically closely related to canine and simian rotaviruses and less so to human M rotavirus (human rotavirus serotype 3). Canine, simian, and human M rotaviruses were closely related. All seven porcine rotavirus strains caused diarrhea in gnotobiotic pigs. Cell-cultured vaccines of the OSU and G strains caused only mild or no diarrhea in gnotobiotic pigs, and protection occurred when such pigs were challenged with homologous, but not heterologous, virulent viruses. A survey indicated that 94% of 274 porcine serum samples and 100% of 75 herds were serologically positive to the porcine OSU rotavirus.

Rotaviruses have been associated with diarrhea in suckling and recently weaned pigs since 1975 (1, 4, 17, 18, 21, 30). Infection is considered to be very prevalent, with most pigs becoming infected during the first few weeks of life (3, 29). There are only a few reports on the cell culture isolation of porcine rotaviruses (2, 8, 25, 26). Isolation or virus replication has been accomplished only by the addition of trypsin or pancreatin to virus suspensions or cell culture media or both and has been facilitated by the use of an established culture of fetal rhesus monkey kidney cells, MA104 (2, 8).

We reported the cell culture isolation of porcine rotavirus, The Ohio State University (OSU) and EE strains, in 1977 (26). Since then, the OSU strain has been widely distributed and reported to be antigenically distinct from other animal and human rotaviruses (see Discussion) and from porcine pararotavirus (5). No report could be found on the antigenic comparison among cell culture-isolated porcine rotaviruses.

This report describes the cell culture isolation of porcine rotaviruses, antigenic comparison of such isolates with each other and with other rotaviruses, studies on cross-protection between porcine rotaviruses in gnotobiotic pigs, and a serological survey in which one porcine rotavirus isolate was used.

MATERIALS AND METHODS

Cell cultures and media. Monolayers of the MA104 cell line and a porcine kidney cell line, PK15, were used. Cells were prepared in flat-bottomed 96- and 24-well microtiter plates (Costar, Cambridge, Mass.), screw-cap tubes (16 by 150 mm), and 2-ounce (60-ml) bottles, as previously described (5). Growth medium was Eagle minimal essential medium supplemented with 10% fetal bovine serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), and mycostatin (25 U/ml). Maintenance medium (MM) was the same as growth medium but did not contain serum. Agar overlay medium for plaque detection was MM supplemented with 0.8% Noble agar (Difco Laboratories, Detroit, Mich.), 0.0007% neutral red, and pancreatin as indicated below.

Pancreatin in cell culture media. Pancreatin was added to agar or liquid medium to facilitate virus replication, plaque formation, or cytopathic effect (CPE), as previously described (5). Briefly, a stock solution composed of 1 volume of pancreatin (4× NF, 2.5% [10×], catalog no. 610-5720; GIBCO Laboratories, Grand Island, N.Y.), and 9 volumes of phosphate-buffered saline (pH 7.2) was added to the cell culture system at a concentration which was slightly less than toxic for the cell monolayer, usually about 1.2% of the stock solution. An alternative procedure which gave comparable results and was usually more convenient consisted of adding drops of pancreatin directly to the cell culture system after the virus absorption period and just before the addition of MM or agar. By this procedure, the amount of pancreatin could be easily varied but was usually as follows: for agar medium in 2-ounce (60-ml) bottles, 3 drops (about 0.1 ml) from a capillary pipette of the stock solution; for 24-well microtiter plates and roller tubes, 3 drops of a 1:40 stock solution per well or tube.

Processing intestinal samples. Large intestinal contents were processed and treated with gentamicin as previously described (5). Cotton swabs were used for swabbing the recta of live pigs, and usually a small amount of adherent feces was collected on each swab. Each swab was inserted into 4 ml of MM, twirled, and removed. The suspension was

* Corresponding author.
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centrifuged, and the supernatant fluid was aspirated and processed as described above. The rectal swab specimen was arbitrarily considered as a 1:25 fecal dilution.

CCIF test. In the cell culture immunofluorescence (CCIF) test, conducted as previously described (5), MA104 cells were used in 96-well microtiter plates. Pancreatin was not added to the cell culture medium; thus, cell-to-cell transmission of infectious rotavirus did not occur, permitting a reliable titration of infectious rotavirus by counting fluorescing-focus units (FFU).

Isolation and propagation of rotaviruses in cell cultures. For isolation purposes, only intestinal specimens with a rotavirus titer of $>10^4$ FFU/ml as judged by the CCIF test were used. The CCIF test was also used in monitoring the extent of rotavirus replication at various cell culture passages, and the results were used to select infected cultures which would be most suitable for further passages. Rotaviruses were isolated by one of the following three techniques. (i) In the plaque technique, 0.2 ml of 1:25, 10^{-2}, and 10^{-4} dilutions of the intestinal specimen was inoculated into each of two culture bottles, so that discernible plaques could be observed in case the virus content was very high. After a virus adsorption period of 60 min at 37°C, culture bottles were rinsed with about 10 ml of MM and one bottle of each dilution was overlaid with agar medium containing pancreatin, whereas the other bottle was treated similarly but contained no pancreatin. Rotavirus plaques occurred only in culture bottles containing pancreatin. Bottles containing typical rotavirus plaques were frozen, and after thawing, the agar and fluid contents were transferred to a tube and centrifuged. The supernatant fluid was used for further cell culture passage. (ii) In the microtiter plate technique, the monolayer in individual wells of a 24-well microtiter plate was inoculated with 0.5 ml of a 1:50 dilution of the intestinal specimen. The plate was centrifuged at 2,800 rpm (1,300 × *g*) for 60 min as previously described (5). The virus inoculum in each well was aspirated and the monolayer was rinsed with 1 ml of MM, after which 1 ml of MM was added. Pancreatin was added to each well as described above, and the plates were incubated at 37°C in a 5% CO_2 atmosphere. After 3 to 4 days, cells and fluid from the wells were collected and used for the next cell culture passage. CPE was evident with some of the specimens after 4 to 8 passages. (iii) The roller tube technique used was similar to that previously described (8, 23). MA104 cells were grown in screw-cap tubes (16 by 150 mm) containing 2 ml of medium in a stationary position for 6 to 8 days. A 0.2-ml amount of a 1:25 dilution of the intestinal specimen was inoculated into each culture tube, incubated at 37°C for 1 h, and rinsed with 2 ml of MM, and 2 ml of MM and pancreatin were added as described above. Culture tubes were incubated at 37°C in a roller drum revolving at about 25 revolutions per h. After 2 to 4 days, the culture tubes were frozen and the cell plus fluid, usually diluted 10^{-1} or 10^{-2}, were used for the next cell culture passage.

Viruses. The sources of the cultivatable reference rotaviruses used in the plaque reduction neutralization (PRN) tests were: canine rotavirus CU-1 from Y. Hoshino, National Institute of Allergy and Infectious Diseases, Bethesda, Md. (13); simian monkey rotavirus MMU 18006 from R. G. Wyatt, National Institute of Allergy and Infectious Diseases, Bethesda, Md., who had obtained it from N. J. Schmidt, California Department of Health Services, Berkeley (24); and human rotavirus Wa (human rotavirus serotype 1) from R. G. Wyatt (32). Human rotavirus M (human rotavirus serotype 3) was obtained from R. G. Wyatt (31) as a 1:50, bacteria-free filtrate from a human stool sample and was adapted to cell culture in our laboratory as described in this report.

The bovine rotavirus used for PRN tests and for preparing antiserum was initially obtained from C. A. Mebus, Plum Island Animal Disease Center, Greenport, N.Y. as the virulent neonatal calf diarrhea virus strain (19). After two passages in gnotobiotic calves in our laboratory, the virus was isolated by the plaque technique with MA104 cells plus pancreatin at a 10^{-7} dilution of gut content. It was subsequently plaque purified once, and most of the PRN tests were conducted with the third or fourth cell culture passage.

The cell culture passage and purification of the other virus strains used in the PRN test were: OSU, passage 60, three plaque purifications; G, passage 34, three plaque purifications; SB-5, passage 33, two terminal dilutions of 10^{-5} each; EE, passage 21; A-580, passage 20, one plaque purification; and M, passage 34.

Animals and antisera. Gnotobiotic pigs were used for experimental infections and production of antisera as previously described (5). Antisera were obtained as convalescent sera 16 to 21 days after oral infection with either cell-cultured adapted viruses propagated in MA104 cells or intestinal contents from gnotobiotic pigs which had been infected with viruses not previously propagated in cell cultures. Antisera prepared with cell-cultured viruses were OSU and G strains of porcine rotavirus, canine rotavirus, and simian rotavirus. Antisera prepared with gut-origin viruses were the EE, A-580, SB-1b (J-1818), SB-3, and SB-5 strains of rotavirus and the Wa and M strains of human rotavirus.

Bovine rotavirus antiserum was prepared in a gnotobiotic pig with a CsCl-purified virulent neonatal calf diarrhea virus strain which was obtained from the intestinal contents of an infected gnotobiotic calf. The virus preparation was administered orally, intramuscularly, and intraperitoneally, with injections every 2 weeks on four occasions. Freund complete adjuvant was mixed with the virus and given on the first injection only. Antiserum was obtained 68 days after initial administration of virus.

PRN test. The PRN test was conducted in MA104 cells grown in 2-ounce (60-ml) bottles as previously described (5). The agar overlay contained pancreatin as described above. The neutralizing antibody titer was expressed as the reciprocal of the initial serum dilution that resulted in an 80% reduction in plaques. Geometric mean titers were given when replicate tests were conducted. Two viruses were considered antigenically distinct when a >10-fold difference in PRN titers occurred when both antisera and viruses were used in a two-way test. Conversely, two viruses were considered antigenically similar when a <10-fold difference in PRN titers occurred when both antisera and viruses were used in a two-way test.

Cross-protection tests. The ability of one porcine rotavirus strain to prevent infection or diarrhea against the same or a different strain was tested in gnotobiotic pigs (see Table 3). Briefly, 2- to 19-day-old pigs were orally exposed to either gut- or vaccine-origin viruses. The gut-origin viruses had been serially passaged only in gnotobiotic pigs and were virulent, that is, capable of causing diarrhea. They were administered orally as 1 ml of a 1:10 sample of intestinal contents containing about 10^5 FFU. The vaccine-origin viruses had been serially passaged in MA104 cells and were considered attenuated because their ability to produce diarrhea was either lacking or reduced. One milliliter of undiluted, cell-cultured virus containing 10^5 to 10^6 FFU was administered orally. These pigs were subsequently exposed to virulent, gut-origin viruses at various time intervals (see Table 3). Pigs were examined at least twice daily for evidence of diarrhea. Rectal swab samples were usually collected daily and titered for rotavirus by the CCIF test.

Electrophoresis of rotaviral ds RNA. Rotaviral double-stranded (ds) RNA was extracted from the intestinal contents of infected gnotobiotic pigs or from infected cell culture medium by CF-11 cellulose chromatography (27). Initially, ds RNA preparations were subjected to electrophoresis in composite 2.5% polyacrylamide–0.5% agarose vertical slab gels and stained with ethidium bromide as described previously (27). In later studies, electrophoresis was conducted in 1.5-mm-thick vertical polyacrylamide slab gels comprising a 4% stacking gel and a 7.5% separating gel. Gels and electrophoresis buffer were prepared according to Laemmli (15), except sodium dodecyl sulfate was omitted; ds RNA was diluted in sample buffer containing 20% (vol/vol) glycerol but no sodium dodecyl sulfate or mercaptoethanol. After electrophoresis at 40 mA for approximately 5 h at room temperature, gels were removed from the glass plates and stained with silver by the method of Herring et al. (11), except sodium borohydride was omitted from the silver reducing solution. Silver-stained gels were photographed by transmitted light with Tri-X film (Eastman Kodak Co., Rochester, N.Y.).

Serological survey. A total of 274 porcine serum samples were obtained from breeding-age swine from 75 Ohio swine herds. Only three to five samples were collected from each herd. These samples had been submitted for routine brucella or pseudorabies serology. They were heat inactivated at 56°C for 30 min and tested at an initial dilution of 1:32 for antibodies against the OSU strain of porcine rotavirus. Samples were considered positive when there was a plaque reduction of more than 80%.

RESULTS

Isolation of rotaviruses in cell cultures. Primary pig kidney cells and an established porcine kidney cell line, PK15, were initially used for isolating the OSU and EE strains of porcine rotaviruses (26). However, MA104 cells were found to be more suitable for this purpose and were used throughout this reported study, except in a few cases, which are specifically noted. Table 1 provides information on the porcine rotaviruses which our laboratory has adapted to cell cultures. Except for strain A-580, cell culture isolations were made from intestinal specimens originating from experimentally infected gnotobiotic pigs. Strain A-580 was isolated directly from intestinal specimens originating from a field case. All seven strains of porcine rotaviruses caused diarrhea in gnotobiotic pigs.

TABLE 1. Porcine rotaviruses adapted to cell culture

Virus strain[a]	Age (days) of pig	Ability to produce plaques on primary isolation	Tentative serotype
OSU	25	Yes	1
EE	11	Yes	1
A-580	23	Yes	1
G	27	No	2
SB-1b	29	No	2(?)
SB-3	33	No	2(?)
SB-5	32	No	2

[a] Strains SB-1b, SB-3, and SB-5 originated from same herd. Other strains originated from different herds.

[b] All were suckling pigs, except for the pig infected with A-580, which had been weaned for 8 days. All pigs had diarrhea.

The plaque technique was very useful for detecting, assaying, and isolating rotaviruses from some intestinal specimens originating from either field cases or experimentally infected gnotobiotic pigs. Viruses which were capable of producing plaques on initial isolation attempts were categorized as biogroup 1 viruses. In contrast, other intestinal specimens which contained high titers ($>10^5$ FFU/ml) of rotavirus as judged by the CCIF test and which originated from either field cases or infected gnotobiotic pigs were negative for plaque formation (Table 1). Viruses which did not produce plaques on initial isolation attempts were categorized as biogroup 2 viruses.

Biogroup 1 viruses included the OSU, EE, and A-580 strains. Titers as high as 10^8 FFU/ml of intestinal or rectal swab samples were occasionally obtained from naturally infected pigs or experimentally infected gnotobiotic pigs. Rotavirus plaques occurred only when the agar overlay contained pancreatin and was usually first observed after 4 to 7 days of incubation. After several cell culture passages, the plaque sizes of the three strains increased. CPE, under liquid media, also readily occurred after 1 to 3 cell passages. Each strain had been passed at least 30 times in MA104 cells. The biogroup 1 viruses so far tested were also found to be antigenically similar and are referred to as porcine rotavirus serotype 1 (see Tables 1 and 2).

Biogroup 2 viruses include the Gottfried (G), SB-1b (J-1818), SB-3, and SB-5 strains. These viruses were more difficult to adapt to cell culture than were those of biogroup 1. The roller tube technique was used for isolation of the SB-1b, SB-3, and SB-5 strains and proved more successful for this purpose than did the microtiter plate technique. Slight evidence of CPE was first observed after three to seven passages in the outgrowth of cells at the periphery of the monolayer in the tube culture. Replicating cells at the periphery of the monolayer seemed more susceptible to rotavirus infection or more apt to express CPE than the more mature cells. With successive cell culture passage, the ability to produce CPE and plaques gradually improved, although good and consistent plaque production by the SB-1b and SB-3 strains was erratic. After 48 to 72 h, 80 to 100% of the cells in infected roller tubes showed CPE, which occurred only in the presence of pancreatin.

Isolation of the G strain deserves special comment, since the specimen from which it was derived appeared to contain a mixture of rotaviruses, one of which was similar to the OSU strain of porcine rotavirus. The intestinal contents of a suckling pig with diarrhea from the Gottfried herd was processed and used to infect a gnotobiotic pig which developed diarrhea and was sacrificed. Intestinal contents were collected and, when examined by the CCIF test, revealed a high titer of rotavirus, but by polyacrylamide gel electrophoresis, the RNA pattern was suggestive of a mixed rotavirus infection. This specimen was then orally administered to a gnotobiotic pig which had recovered from infection with the OSU strain of porcine rotavirus. After about 24 h, the pig was sacrificed and specimens were collected. The intestinal contents revealed a high titer of rotavirus by the CCIF test, and the electrophoretic migration pattern of RNA suggested the presence of only one rotavirus whose RNA pattern was distinct from that of the OSU strain. Subsequent passages of this virus in gnotobiotic pigs and in cell cultures indicated an absence of the OSU type of rotavirus and the presence of only one virus. With a high-titered rotavirus ($>10^6$ FFU/ml) intestinal sample from an infected gnotobiotic pig, the G strain was serially passed six times in PK15 cells and five times in MA104 cells, by the microtiter plate technique. Replication as judged by the CCIF test occurred at each passage level, but CPE was slight or negligible. With subsequent passages in MA104 cells, in which either 24-well plates or 2-ounce (60-ml) bottles were used, the ability of the virus to produce CPE and plaques gradually improved. CPE and plaques occurred only in the presence of pancreatin.

The human rotavirus, M strain, was also adapted to cell culture by the roller tube technique with intestinal contents (titer, 10^7 FFU/ml) from the fourth serial passage in gnotobiotic pigs. CPE and plaque production improved on serial passage.

PRN test. The results of the PRN test are shown in Table 2. Porcine rotavirus strains OSU, EE, and A-580 were antigenically very similar, if not identical, and were categorized as porcine rotavirus serotype 1. The OSU strain, as the prototype virus, was antigenically distinct from porcine rotavirus strains G and SB-5 and from bovine, simian, canine, and human (Wa and M strains) rotaviruses.

Porcine rotavirus strains G and SB-5 were antigenically similar and were categorized as porcine rotavirus serotype 2. Both strains were antigenically distinct from the OSU strain (serotype 1). The SB-1b and SB-3 strains may also be candidates for serotype 2, since their antisera neutralized the G and SB-5 strains but not the OSU strain. Since the use of the SB-1b and SB-3 viruses in the PRN test has so far resulted in erratic plaque counts, such data were deemed unreliable and are not reported.

Of special note was the cross-neutralization occurring among the porcine G, porcine SB-5, canine, simian, and human M rotaviruses, indicating antigenic relatedness. To assist in evaluating the degree of cross-neutralization, the neutralization ratio (NR), that is, the ratio of the titer of the antiserum with heterologous virus to that of the antiserum with homologous virus, is given. For porcine G strain antiserum, the NR was about 1 with canine, 1/4 with simian, and 1/12 with human M rotaviruses. For simian rotavirus antiserum, the NR was about 1 with both canine and human M and 1/2 with G strain rotaviruses. For canine rotavirus antiserum, the NR was about 1/4 for all three heterologous viruses. For human M strain antiserum, the NR was about 1/2 with G strain, 1/4 with canine and simian, and 1/6 with porcine SB-5 strain rotaviruses.

Although the G and SB-5 strains were antigenically similar, the reactivity of their antisera with those of other rotavirus strains differed rather markedly. For example, G strain, but not SB-5 strain, antiserum showed significant neutralization of simian and canine rotaviruses (NR, ≤1/10).

Hyperimmune bovine rotavirus antiserum had an NR of about 1/4 with canine, 1/8 with human M, 1/15 with porcine G, and 1/16 with simian rotaviruses; however, bovine rotavirus was not significantly neutralized by any of the heterologous antisera. Human rotavirus Wa antiserum neutralized the porcine SB-5 strain at a higher titer than it did the homologous virus; however, SB-5 antiserum did not significantly neutralize the Wa strain.

Cross-protection among porcine rotaviruses in gnotobiotic pigs. The OSU vaccine strain, after 35 serial passages in MA104 cells, produced only slight evidence of diarrhea when administered to 3- to 5-day-old pigs. (Table 3, pigs 1 through 5). When pigs 1, 2, and 3 were challenged 14 or 22 days later with the OSU gut strain, neither diarrhea nor virus excretion was detected. Pigs 2 and 3 had a third virus exposure to the G and SB-1b strains, respectively, resulting in virus excretion and diarrhea. Pigs 4 and 5 were challenged with the G strain, resulting in virus excretion, but diarrhea was either absent or questionable.

Previous exposure of pigs to the OSU gut strain did not prevent diarrhea or virus excretion when challenged with the G strain (pigs 6 and 7) but did when challenged with the homologous strain (pig 8). Fecal excretion of virus, but not diarrhea, was detected in pigs 9, 10, and 11, which had been initially exposed to the OSU strain and challenged with the SB-1b, SB-5, and SB-3 strains, respectively.

Exposure of 3- to 5-day-old pigs to the G vaccine strain, serially passed 17 or 28 times in MA104 cells, resulted in an absence or only slight diarrhea (Table 3, pigs 12 through 15). When challenged with homologous gut virus, neither diarrhea nor virus excretion was detected (pigs 12 and 13), but

TABLE 2. Antigenic relationships of selected rotaviruses

Rotavirus strain	PRN antibody titers obtained with antisera to:											
	Porcine							Bovine[c]	Simian[a]	Canine[a]	Human	
	OSU[a]	EE[b]	A-580[b]	G[a]	SB-1b[b]	SB-3[b]	SB-5[b]				Wa[b]	M[b]
Porcine												
OSU	**390**	1,152	210	<4	<4	<4	<4	120	4	<4	<4	<4
EE	270	720	400	4	—[d]	—	<4	—	25	—	—	—
A-580	430	1,400	**256**	<4	5	4	<4	—	5	5	—	5
G	6	20	9	**1,500**	133	300	310	410	240	350	83	620
SB-5	34	—	20	560	450	3,000	**1,200**	—	26	94	820	220
Bovine	6	13	<4	<4	<4	<4	<4	**6,200**	5	7	<4	<4
Simian	5	18	8	400	—	—	10	370	**440**	370	33	310
Canine	5	25	68	1,024	5	<4	<4	1,700	340	**1,300**	72	360
Human												
Wa	4	—	5	27	5	—	13	180	5	7	**440**	74
M	<4	—	21	120	<4	8	14	800	440	430	86	**1,300**

[a] Convalescent serum from gnotobiotic pig infected with cell-cultured virus.
[b] Convalescent serum from gnotobiotic pig infected with gut-origin (virulent) virus.
[c] Hyperimmune serum from gnotobiotic pig with gut-origin virus from an infected gnotobiotic calf.
[d] —, Not tested.

TABLE 3. Cross-protection among porcine rotavirus isolates in gnotobiotic pigs

Pig no.	Virus exposure											
	1st				2nd				3rd			
	Virus[a]		Pig age (days)	Diarrhea[b]	Virus (gut)	Pig age (days)	Fecal excretion (FFU/ml)[c]	Diarrhea[b]	Virus (gut)	Pig age (days)	Fecal excretion (FFU/ml)[c]	Diarrhea[b]
	Gut	Vaccine										
1		OSU (35)	5	+ (sl)	OSU	19	—	−				
2		OSU (35)	5	+ (sl)	OSU	19	—	−	G	33	$>10^4$	+
3		OSU (35)	3	+ (sl)	OSU	25	—	−	SB-1b	38	$>10^5$	+ (sl)
4		OSU (35)	3	+ (sl)	G	25	$>10^4$	−				
5		OSU (35)	3	+ (sl)	G	25	$>10^4$	?				
6	OSU		10	+	G	32	$>10^4$	+				
7	OSU		10	+	G	32	$>10^4$	+				
8	OSU		7	+	OSU	26	—	−				
9	OSU		7	+	SB-1b	26	$>10^4$	−				
10	OSU		8	+	SB-5	30	$>10^4$	−				
11	OSU		19	+	SB-3	47	$>10^6$	−				
12		G (17)	5	−	G	19	—	−	OSU	33	$10^{5.5}$	+ (sl)
13		G (17)	5	−	G	19	—	−	OSU	33	$10^{3.5}$	+ (sl)
14		G (28)	3	+ (sl)	OSU	25	$>10^5$	+ (sl)				
15		G (28)	3	?	OSU	25	$>10^5$	+ (sl)				
16	G		16	+	G	41	—	−				
17	G		16	+	G	41	—	−				
18	G		16	+	OSU	41	$>10^4$	+ (sl)				
19	G		16	+	OSU	41	$>10^4$	+ (sl)				
20	G		19	+	SB-3	47	—	−				
21	SB-1b		7	+	SB-1b	26	—	−				
22	SB-1b		7	+	OSU	26	$>10^4$	+ (sl)				
23		SB-5 (12)	5	+ (sl)	SB-5	26	—	−	OSU	40	$10^{6.3}$	+
24	SB-5		2	+	OSU	22	$10^{6.4}$	+				

[a] Gut virus (virulent) indicates the immediate source of virus and that it was passed only in gnotobiotic pigs. Vaccine virus indicates that the virus was replicated in cell culture. Numbers in parentheses indicate cell culture passages.

[b] +, Diarrhea; + (sl), slight diarrhea; ?, questionable diarrhea; −, no diarrhea observed.

[c] FFU titers are expressed as FFU per milliliter and represent the highest titers obtained during a 24- to 72-h postexposure period. —, No virus detected when 0.2 ml of approximately 1:25 rectal swab suspension was used.

when challenged with the OSU strain, virus excretion and slight evidence of diarrhea were detected (pigs 12 through 15).

Exposure of 16- or 19-day-old pigs to the G gut strain resulted in diarrhea (pigs 16 through 20). Challenge with the homologous virus 25 days later (pigs 16 and 17) or with the SB-3 strain 28 days later (pigs 20) resulted in no detectable diarrhea or virus excretion, whereas challenge with the OSU strain (pigs 18 and 19) resulted in virus excretion and slight diarrhea. The SB-1b gut strain provided protection against challenge with homologous virus (pig 21) but not against the OSU strain (pig 22).

Exposure of a 5-day-old pig (pig 23) to the 12th cell culture passage of the SB-5 strain resulted in slight evidence of diarrhea. When challenged with homologous gut virus 21 days later, neither diarrhea nor virus excretion was detected, but on the third virus exposure to the OSU strain, diarrhea and virus excretion occurred. Exposure of pig 24 to the SB-5 gut virus did not prevent diarrhea or virus excretion after challenge with the OSU strain.

Electrophoresis of rotaviral ds RNA. The porcine rotavirus strains OSU, G, and SB-5 and the human rotavirus strains Wa and M could be readily distinguished by differences in the electrophoretic migration patterns (electropherotypes) of their ds RNA (Fig. 1). The electropherotypes of the cell culture-passaged porcine rotavirus strains OSU, G, and SB-5 were identical to those of their respective gut viruses from which they were derived.

Serological survey. Of 274 porcine serum samples tested for antibodies against the OSU strain of porcine rotavirus, 257 (94%) were positive, and each of the 75 herds contained serologically positive animals.

DISCUSSION

Rotaviruses isolated from pigs in this study could be divided into two biogroups on the basis of the ability to produce plaques on initial cell culture isolation attempts with MA104 cells and pancreatin in the agar medium (Table 1). Biogroup 1 contained the OSU, EE, and A-580 strains, which produced plaques. These viruses also replicated well and produced CPE in MA104 cells when the medium contained pancreatin. Biogroup 2 contained the G, SB-1b, SB-3, and SB-5 strains, which did not produce plaques on initial isolation attempts and which were much more difficult to adapt to cell cultures. The roller tube procedure, in which MA104 cells and pancreatin were used, appeared to be the procedure of choice for the cell culture isolation of these viruses. Isolation of porcine (8) and human (23, 28) rotaviruses by this procedure has been reported previously.

Rotaviruses isolated from pigs could be antigenically divided into at least two serotypes. The OSU, EE, and A-580 strains, all members of biogroup 1, were very similar, if not identical. We suggest that these viruses be considered as porcine rotavirus serotype 1, with the OSU strain serving as the prototype virus. The OSU strain was antigenically distinct from porcine rotavirus strains G and SB-5. Our results agree with those of previous reports that the OSU strain is antigenically distinct from bovine (5, 7, 9, 22), canine (9), simian (9), and human Wa (32) rotaviruses. We

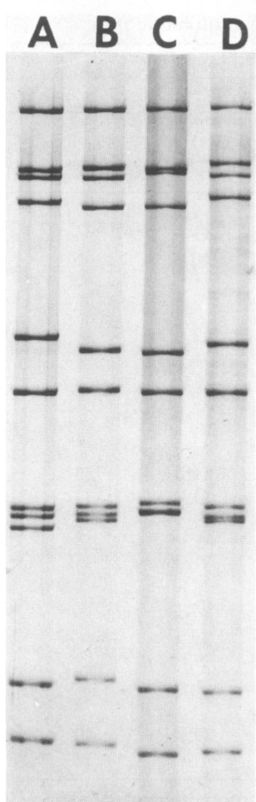

FIG. 1. Silver strain of rotaviral ds RNA resolved in a Laemmli polyacrylamide gel. Migration is from top to bottom. Lane A, human rotavirus, Wa strain; lane B, human rotavirus, M strain; lane C, porcine rotavirus, OSU strain; lane D, porcine rotavirus, G strain. All RNA preparations were derived from intestinal contents of infected gnotobiotic pigs.

found it to be distinct from human M rotavirus. Sato et al. (22) reported it to be distinct from lapine rotavirus.

Strains G and SB-5, members of biogroup 2, were antigenically similar. We suggest that they be considered as porcine rotavirus serotype 2, with the G strain tentatively designated as the prototype virus. Both strains were antigenically distinct from the OSU strain. Although the G and SB-5 strains were antigenically similar, comparison with other rotaviruses revealed marked differences. For example, with canine rotavirus, G strain antiserum reacted at a titer of 1,024, whereas SB-5 strain antiserum had a titer of <4.

Our results indicated a two-way antigenic similarity of the porcine G with simian and canine rotaviruses and a one-way similarity of the porcine G with the human M rotavirus. We also found a two-way antigenic similarity of the human M, simian, and canine rotaviruses. Others have reported the similarity of the simian and human M rotaviruses (31) and the canine and simian rotaviruses (9, 12, 13). A one-way antigenic relationship between the canine and human M rotaviruses has been reported previously (12), whereas our findings suggest a two-way relationship. Hoshino et al. (13) have reported a one-way relationship of the canine with bovine and porcine OSU rotaviruses, whereas our results confirm the former but not the latter.

Rotaviruses have also been antigenically categorized by tests other than neutralization. By this method, OSU and EE strains belong to subgroup 1 (10, 14) and the G strain belongs to subgroup 2 (10). The last finding is of special note, since only two rotavirus strains of animal origin (G strain and an equine strain) have been detected with subgroup 2 specificity, whereas all others were of human origin (10). A more thorough comparison of the porcine G and human M rotaviruses is indicated because of the similarities in their serotype and subgroup specificities.

There appears to be a common sharing of antigens among rotaviruses, allowing for various and complex patterns of cross-reactivity. Subtle differences in procedures used for preparing virus or antiserum reagents can markedly influence results, as others have indicated (7, 9, 31) and as we have found (data not shown). For the PRN tests, we generally used early convalescent antiserum from orally infected gnotobiotic pigs. Recently, however, the trend has been to use hyperimmune antiserum prepared in guinea pigs or rabbits by injections of semipurified rotaviruses (9, 12, 13, 23, 31). Convalescent antiserum has broader antigen specificity than hyperimmune antiserum (7, 9, 31), which could account for the minor discrepancies observed between our results and those of others who used hyperimmune antiserum.

Cell culture-passaged or vaccine strains OSU, G, and SB-5 appeared to be less capable of causing diarrhea than did the same strains which had been passaged only in gnotobiotic pigs (Table 3). When pigs were vaccinated with these three strains and later challenged with homologous, virulent virus, neither diarrhea nor virus excretion was detected. Thus, passage in cell culture had retained the antigenic characteristic of the strain. Such strains may be of value as vaccines for immunizing either piglets or pregnant swine, the latter for providing an increased level of passive immunity to suckling pigs. Gaul et al. (9) have also reported that a vaccine of the OSU cell culture-adapted strain originating from our laboratory protected gnotobiotic pigs challenged with the virulent OSU virus. They also reported that prior infection with bovine, canine, or simian rotavirus did not protect gnotobiotic pigs from diarrhea or virus excretion when challenged with the virulent OSU virus.

For the development and use of rotavirus vaccines for immunizing swine, consideration should be given to the fact that there are at least two porcine rotavirus serotypes and that complete cross-protection between heterologous serotypes will probably not be provided. A porcine rotavirus vaccine containing the cell culture-adapted porcine OSU rotavirus has been federally licensed and is commerically available (C. J. Welter, Ambico, Inc., Dallas Center, Iowa, personal communication). Cross-protection studies indicated differences between the following pairs of porcine rotavirus strains: OSU and G, OSU and SB-1b, OSU and SB-3, and OSU and SB-5 (Table 3). However, in some cases, infection with one virus prevented or reduced the severity of diarrhea in pigs challenged with heterologous strains, although virus excretion occurred (Table 3). This may be partly owing to the older age at which some of the pigs were challenged, since more severe diarrhea generally occurs in younger pigs, or to partial protection afforded by previous infection with antigen-related viruses. One pig infected with the G strain was protected against diarrhea and virus excretion when challenged with the SB-3 strain, suggesting a close antigenic relationship. More serological and cross-protection studies are necessary for the porcine rotavirus strains G, SB-1b, SB-3, and SB-5 to determine their proper relationship among themselves and with other rotaviruses.

The electropherotypes of the porcine rotavirus strains OSU, G, and SB-5 and human rotavirus strains Wa and M further confirmed their separate identities, as also shown by PRN or cross-protection tests or both. The electropherotypes of the cell culture-adapted OSU, G, and SB-5 strains were identical to those of their respective gut viruses from which they were derived, further indicating the stability of the virus during cell culture passage and the probable absence of extraneous rotaviruses.

A serological survey indicated the high prevalence of rotavirus infections in swine, as has been reported by others in the United States (16), the Federal Republic of Germany (1), France (6), and Hungary (20).

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